REMARKS

The foregoing amendments rewrote claim 30 as an independent claim including the limitations of claim 27 from which it previously presented. In addition, the foregoing amendments canceled claims 42, 43, and 45-48 and amended claims 44 and 49 to depend from independent claim 27. The amendments included editorial changes to claims 37, 38, and 40. The foregoing amendments were made to clarify what was already implied in applicant's claims and these amendments are not narrowing amendments and were not made for reasons substantially related to patentability presented. Claims 27-30, 32-40, 44, and 49 remain in the application for consideration by the examiner.

The Official action set forth a single prior art rejection of claims 27-30, 32-40 and 42-49 under 35 U.S.C. §103(a) as being unpatenable over U.S. Patent No. 4,643,902 of Lawhon *et al.* ("Lawhon") in view of Nakhmedov *et al.* (Koservanaya I Ovoshchesushil'naya Promyshlennost, hereinafter referred to as "Nakhmedov") and British Patent Specification No. 1,007,751 ("British '751"). This rejection is similar to that set forth the previous Office action. In the outstanding Office Action, the rejection was expanded on pages 3-9 of the Official action. In addition, the Official action included a "Response to Arguments" on pages thereof. Applicant respectfully submits that the presently claimed inventions are patently distinguishable from the teachings of Lawhon, Nakhmedov, and British '751 for the reasons set forth in the previously filed responses, including that filed on September 29, 2006, which are incorporated herein by reference. Applicant also submits that the inventions defined in the present claims are patentable from the teachings of Lawhon, Nakhmedov, and British '751 for at least the following reasons.

The positions proffered in the Official action and their locations in the Official action are summarized below.

(1) *Amount of anthocyanin in all claims, Official action page 3, bottom.* The Official action commented that Nakhmedov teaches in Table 1, a black currant composition comprising 6256.8 ± 11.5 mg/100 g and 6128.9 ± 15.2 mg/100 g, which corresponds to approximately 6.26% that is encompassed by the presently claimed amount of 5 to 25% of black currant anthocyanin, and such a composition meets the requirements of applicant's claim because there is no monosaccharide contained therein and there is no organic acid mentioned therein.

Applicant's response:

The attached scheme, which is labeled as "Attachment 1," shows the process of purifying a coloring agent proposed in Nakhmedov. In the process, the raw material is pressed and a part other than juice is used as pressed skin, which is usually treated as a waste, to purify the coloring agent. The pressed skin is divided into two parts. The anthocyanin content of the first part of the pressed skin is analyzed as the firstly pressed marc and the result is disclosed in Table 1 (Contents in marcs, of the first pressing). The second part of the pressed skin is further pressed and analyzed as the secondarily pressed marc and the result is disclosed in Table 1 (Content in marcs, of the second pressing). The firstly pressed marc and secondarily pressed marc are further treated to separate juice, and the coloring agent is extracted with hot water. The anthocyanin content of the coloring agent from the firstly pressed mark is disclosed in Table 1 (Quantity of coloring agent produced from marc, of the first pressing). The anthocyanin content of the coloring agent from the secondarily pressed mark is also disclosed in Table 1 (Quantity of coloring agent produced from marc, of the second pressing). The result of detailed analysis including the content of monosaccharide and acidity of the coloring agent is disclosed in Table 3 -- this data is for the same marks as in Table 1 of Nakhmedov. Thus, the teachings of Nakhmedov propose that the marcs proposed therein necessarily contain monosaccharides and

organic acids, and therefore, these teachings cannot contemplate or suggest a composition not containing monosaccharides and organic acids, as presently claimed.

(2) *Amount of monosaccharide in all claims, Official action page 4, middle and bottom and page 9, bottom.* The Official action took the position that because no monosaccharide is described in Table 1 of Nakhmedov, there is no monosaccharide contained in the marcs described therein.

Applicant's response:

This position is incorrect and disregards the overall teachings of Nakhmedov. Table 1 of Nakhmedov was created only to show the information therein, such as only the anthocyanin and coloring agent content in marcs, and not to obscure this showing by providing additional information containing organic acids, monosaccharides, etc. Black currant necessarily contains organic acids and monosaccharides, as understood by any person skilled in the art and as taught by Nakhmedov. This is evidenced by applicant's (Hitoshi MATSUMOTO *et al.*) specification disclosure and cited prior art where the organic acid and monosaccharide content of black currant berries are disclosed. In particular, the present application describes "Fresh juice just squeezed from black currant ... contains about 20 to 30% by weight of organic acids such as citric acid and malic acid on the basis of solid matters, and about 30 to 50% by weight of monosaccharide on the basis of solid matters" at lines 16 to 19 on page 1. Table 3 of Nakhmedov shows supplemental information about the *same marcs* as Table 1 of Nakhmedov, such as organic acid and monosaccharide content. Table 3 of Nakhmedov clearly shows that colors from black currant marc necessarily contain 16.1 ± 0.7% sugars. It would be understood by those skilled in the art that these sugars contain significant amount of monosaccharides.

The Official action alleged that the sugar content shown in Table 3 of Nakhmedov can be monosaccharides, disaccharides and complex carbohydrates and concluded that absent evidence to the contrary, Nakhmedov teaches a sugar, not a monosaccharide. However, from the above, applicant respectfully submits that any person skilled in this art would understand that the sugars shown in Table 3 of Nakhmedov necessarily contain a monosaccharide. This conclusion is supported by the attached article, Boccorh *et al.*, entitled *Factors influencing quantities of sugars and organic acids and blackcurrant concentrates* in Z Lebensm Unters Forsch A (1998) 206; 273-278, which is labeled "Attachment 2," and which discloses the sugar contents of black currant concentrate in Table 1 thereof. Table 1 of Attachment 2 clearly shows that the main sugars of black currant concentrate are fructose and glucose -- monosaccharides. See also, the attached article of Sanna Viljakainen, entitled *Reduction of Acidity in Northern Region Berry Juices* in ISBN 951-22-6435-8 (2003), which is labeled "Attachment 3." From this information, those skilled in the art would understand that the composition of marcs disclosed in Table 1 of Nakhmedov must necessarily contain organic acids and monosaccharides, in contrast to the present claims.

Put another way, according to the examiner's calculations set forth at the bottom of page 3 of the Official action, Table 1 of Nakhmedov shows about 6.26% anthocyanin. Those skilled in the art understand that the remaining 73.74% of the composition of the marcs disclosed in Table 1 of Nakhmedov must necessarily contain organic acids and monosaccharides.

For all these reasons, applicant respectfully submits that any person skilled in this art would understand that the marcs proposed by Nakhmedov must necessarily contain organic acids and monosaccharides, in contrast to the presently claimed invention. Therefore, applicant

respectfully requests that the examiner reconsider and withdraw the rejection of the present claims over the teachings of Nakhmedov combined with Lawhon and British '751.

(3) *Amount of organic acids in all claims, Official action page 4 top, page 9 bottom and page 10 top.* The Official action took the position that because no acid is described in Table 1 of Nakhmedov, there is no acid contained in the marcs described therein.

<u>Applicant's response</u>:

The Official action made similar arguments with respect to the presently claimed amount of no monosaccharide, and applicant's position for responding thereto is the same as set forth above in position (2). Namely, Table 1 of Nakhmedov was created only to show limited information therein, such as only the anthocyanin and coloring agent content in marcs, and not to obscure this showing by providing additional information containing organic acids, monosaccharides, etc. Black currant necessarily contains organic acids and monosaccharides, as understood by any person skilled in the art and as taught by Nakhmedov. Table 3 of Nakhmedov shows *the same black currant marcs* have 9.8 ± 0.2% of total acidity. Moreover, Attachment 3, "Sanna Viljakainen, entitled *Reduction of Acidity in Northern Region Berry Juices* in ISBN 951-22-6435-8 (2003)," discusses that the main acids of black currant berry juices were invariably citric and malic acid. Furthermore, those skilled in the art understand that the total acidity means the content of organic acids, because Nakhmedov refers to the content of organic acids and sugars in the marc at the 9th line from the bottom on page 6 to the 6th line from the bottom on page 6 of the English translation of Nakhmedov, which was attached to applicant's response filed on April 23, 2004. From the above, applicant respectfully submits that it is incontrovertible that

the marcs in Table 1 of Nakhmedov necessarily contain amounts of organic acids in excess of that required in the present claims.

Applicant respectfully submits that it is impermissible within the framework of 35 U.S.C. §103 to select a single line or two of a reference (i.e., only Table 1 of Nakhmedov) in total disregard for the remaining teachings of the reference (i.e., Table 3 of Nakhmedov) and then rely upon the reference with the benefit of hindsight to show obviousness. *Bausch & Lomb, Inc. v. Barnes-Hind/Hydrocurve, Inc.*, 230 U.S.P.Q. 416, 419 (CAFC 1986). It has long been held that it is impermissible within the framework of §103 to pick and choose from any one reference only so much of it as will support a given position to the exclusion of other parts necessary to the full appreciation of what such reference fairly suggests to one skilled in the art. *In re Wesslau*, 147 U.S.P.Q. 391, 393 (CCPA 1965); *In re Mercer*, 185 U.S.P.Q. 774, 778 (CCPA 1975). For these reasons, applicant respectfully submits that the teachings of Nakhmedov either alone or combined with Lawhon and British '751 cannot contemplate or suggest a composition containing a limited amount of organic acid and no monosaccharide, as presently claimed. Therefore, applicant respectfully requests that the examiner reconsider and withdraw the rejection of these claims over the teachings of Nakhmedov, Lawhon, and British '751.

None of the cited teachings contemplates or suggests the amount of delphinidin and delphinidin-3-0-rutinoside, required for example, in present claims 28 and 29, together with the other requirements of these claims. While the presence of delphinidin or delphinidin-3-0-rutinoside may have been known in black currant, which the applicant does not admit, an important characteristic of the presently claimed invention is a composition containing a high content of delphinidin and delphinidin-3-0-rutinoside as a food composition (i.e., having low amounts of monosaccharides and organic acids). The teachings of Nakhemedov are concerned

with obtaining a dye. In contrast, the presently claimed invention is directed to obtaining a food composition (substantially free of monosaccharide and having low content of organic acid). Therefore, applicant respectfully submits that the teachings of Nakhemedov can provide a person of ordinary skill in the art with no reason to obtain the presently claimed food composition. Furthermore, Nakhemedov never suggests the content of delphinidin-3-0-rutinoside, and therefore cannot contemplate or suggest the presently claimed amount of delphinidin-3-0-rutinoside, etc.

(4) *Product-by-process limitations and negatively charged membrane, Office action page 4 bottom.* The Official action never explained where the teachings of the cited references contemplate or suggest, *inter alia*, the use of a negatively charged reverse osmosis membrane to purify and concentrate the black currant juice, as required in claims 30 and 44 and the claims that depend thereon. With respect to claims 30, 32, 33, 34, the Official action stated that these claims are product-by-process claims and the patentability of a product does not depend on its method of production.

Applicant's response:

Present claims 30, 32, 33, and 34 are not product-by-process claims, but rather are process claims. Accordingly, the comments in the Official action concerning product-by-process claims are not pertinent to these claims. Applicant respectfully submits that claims 30, 32, 33, and 34 are patently distinguishable from the teachings of Nakhmedov, Lawhon, and British '751 within the meaning of 35 U.S.C. §103 for the reasons set forth in the previously filed responses and for at least the following reasons.

The foregoing amendments rewrote claim 30 as an independent claim including the limitations of claim 27. For example, claim 30 was amended to define a composition comprising

5 to 25% by weight of black currant anthocyanin and an organic acid content of not more than 5% by weight on the basis of solid matters, and monosaccharide is not found.

The teachings of Lawhon propose the purification and concentration of juice by using a reverse osmosis membrane. In the process disclosed in Lawhon using reverse osmosis (RO) membrane, sugars and acids are fractionated in a RO retentate together with anthocyanin. In contrast thereto, the membrane used for the presently claimed invention is a *negatively charged* reverse osmosis membrane. In the process of the presently claimed invention using the *negatively charged* RO membrane, anthocyanin is fractionated in a retentate however sugars and acids are separately fractionated in a permeate -- an arrangement opposite to that proposed by Lawhon.

The RO membrane, such as proposed by Lawhon, was developed for desalinating seawater to obtain fresh water. The RO membrane is placed between seawater and fresh water. When a pressure higher than the pressure of osmotic pressure is applied to seawater, water in the seawater is moved to the fresh water through the RO membrane. Since the retention rate of NaCl of the RO membrane is more than 99%, fresh water can be obtained by using the RO membrane. In contrast thereto, the charged RO membrane was developed separately from the RO membrane as a nanofilter (or a loose RO membrane). The size of a substance removed by the charged RO membrane is between that of the RO membrane and ultrafiltration (UF) membrane. That is, the charged RO membrane operates and functions differently from a RO membrane as a filter, as understood by those skilled in the art. Therefore, the teachings of Lawhon cannot possibly provide a reason to one of ordinary skill in the art to use a process proposed therein in combination with a negatively charged reverse osmosis membrane, as required in present claim 30. The teachings of Nakhmedov and British '751 do not cure or rectify this deficiency in the

teachings of Lawhon. At least for these reasons, applicant respectfully submits that the invention defined in independent process claim 30 and the claims that depend thereon are patently distinguishable from the teachings of Nakhmedov, Lawhon, and British '751. Therefore, applicant respectfully requests that the examiner reconsider and withdraw the rejection of claims 30, 32, 33, and 34 that was set forth in the outstanding Office action.

Claim 44 is a product-by-process claim. Applicant respectfully submits that the present specification disclosure establishes the importance and significance of the method steps set forth in claim 44, and moreover, establishes that the product resulting from the product-by-process steps set forth therein results in a patently distinguishable product. Namely, in the presently claimed invention, the organic acids content can be reduced without using an ion exchange column. Example 1 of the present application shows that the use of the negatively charged reverse osmosis membrane itself accomplished the reduction of organic acids and the delction of monosaccharide. As shown in Example 2 of the present application, the ion exchange column was used to increase anthocyanin content, not to reduce the organic acids content. These examples show the differences between the use of a negatively charged reverse osmosis membrane and an ion exchange column, especially with respect to the presently claimed invention, and that they cannot be interchangeably used to arrive at the compositions defined in the present claims. For such reasons, applicant respectfully submits that the invention defined in claim 42 is patently distinguishable from the teachings of Nakhmedov, Lawhon, and British '751. Therefore, applicant respectfully requests that the examiner reconsider and withdraw the prior art rejection set forth in the outstanding Office action.

(5) *Intended use, Office action page 5, middle and bottom.* The Official action cited some case law explaining there is no general rule as to the weight given a preamble as a positive limitation affecting the patentability of the claimed subject matter. The Official action stated that the intended use must result in a structural difference between the claimed invention and the prior art in order to patently distinguish the claimed invention from the prior art.

Applicant's response:

Page 9 of the response filed on September 29, 2006, which remarks are incorporated herein by reference, explained why the teachings of Nakhmedov cannot disclose or suggest the inventions defined in, for example, claim 27 that are directed to "A black currant anthocyanin-containing *food composition suitable for human consumption*." The Official action provided no reasons why the arguments provided in the response are not correct, but simply stated that the marcs of Nakhmedov are capable performing the intended use of the present claims. Applicant respectfully submits that this position is incorrect for least the following reasons.

The teachings of Lawhon are not concerned with concentrating black currant juices and the methods proposed therein cannot obtain a composition having anthocyanin, organic acid and monosaccharide concentrations as presently claimed. As discussed above, the teachings of Nakhmedov are concerned with preparing a coloring agent. Since the teachings of Lawhon and Nakhmedov have nothing to do with a "black currant anthocyanin-containing *food composition suitable for human consumption*," and, in fact, teach away from such a composition, applicant respectfully submits that these teachings either alone or combined with other teachings cannot provide any reason to one of ordinary skill in the art prepare a "black currant anthocyanin-containing *food composition suitable for human consumption*," as presently claimed.

With respect to present claims 35 and 36, the presently claimed food or drink includes the black currant anthocyanin-containing food composition according to claim 27. As explained above, the black currant anthocyanin-containing food composition according to claim 27 has structural differences from the prior art. For at least this reason, the presently claimed food or drink of claims 35 and 36 is patently distinguishable from the prior art.

Concerning present claims 37 to 40, the claimed black currant anthocyanin-containing food composition according to claim 27. Claims 37 and 39 that depend on claim 27 are directed to a food composition. As mentioned above, the claimed composition of claim 27 is different from the composition disclosed in Nakhmedov, Lawhon, and British '751. For at least this reason, the presently claimed food or drink of claims 37 and 39 is patently distinguishable from the prior art.

Present claims 38 and 40 depend on claim 35, which depends on claim 27, and are directed to a food or drink. As mentioned above, the claimed composition of claim 27 is different from the composition disclosed in Nakhmedov, Lawhon, and British '751. For at least this reason, the presently claimed food or drink of claims 38 and 40 is patently distinguishable from the prior art.

Furthermore, the food composition of present claims 37 and 39, and the food or drink of present claims 38 and 40 contain anthocyanin amount that accomplishes the properties recited in each claim. That is, the properties recited in present claims 37 to 40 patently distinguish the claimed composition and food or drink from the different composition proposed by Nakhmedov, Lawhon, and British '751. For least this reason, the presently claimed food compositions of claims 37 and 39 and foods or drinks of claims 38 and 40 are clearly different from the composition disclosed in Nakhmedov, Lawhon, and British '751.

(6) *The references cannot properly be combined.* The Official action stated that the references can properly be combined because they both refer to a common problem of concentrating fruit juice.

Applicant's response:

The teachings of Nakhmedov are not concerned with concentrating fruit juice. Namely, the teachings of Nakhmedov are directed to the production of coloring agent from marcs (waste) of black currant. The teachings of Nakhmedov never contemplate or suggest concentrating fruit juice, as presently claimed. An important aspect of the presently claimed invention is reducing the concentration of monosaccharide and organic acids in a composition made from black currant. The teachings of Nakhmedov never suggest the reduction of monosaccharide and organic acids. Therefore, the teachings of Nakhmedov can provide no reason to those skilled in the art to modify the process of Lawhon so as to arrive at the presently claimed composition that have specific amounts of anthocyanin, organic acid, and no monosaccharide.

Furthermore, the teachings of Nakhmedov never disclose or suggest the presently claimed composition. Similarly, the teachings of Lawhon never disclose or suggest the claimed process of the present application. The teachings of British '751 do not cure or rectify these deficiencies in the teachings of Nakhmedov and Lawhon. Accordingly, it is impossible for one of ordinary skill in the art to review the combined teachings of Lawhon, Nakhmedov, and British '751 and have a reason to prepare the compositions of applicant's claims. For at least this reason, applicant respectfully submits that the inventions defined in claims 27-30, 32-40, 44, and 49 are patently distinguishable from the combined teachings of Lawhon, Nakhmedov, and British '751

within the meaning of 35 U.S.C. §103. Therefore, applicant respectfully requests that the examiner reconsider and withdraw this rejection.

In summary, the Official action apparently took the position that Nakhmedov teaches the amounts of solids left from processing black currant berries, as proposed therein, and combining this knowledge with the process as proposed by Lawhon will lead to applicant's claimed invention. However, as explained above, even if one of ordinary skill in the art did this, the teachings of Nakhmedov provide no reason to one of ordinary skill in the art to modify the process proposed by Lawhon so as to arrive at the presently claimed compositions that have specific amounts of anthocyanin, organic acid, and no monosaccharide. For at least these reasons, applicant respectfully submits that the inventions defined in claims 27-30, 32-40, 44, and 49 are patently distinguishable from the combined teachings of Lawhon, Nakhmedov, and British '751 within the meaning of 35 U.S.C. §103.

For at least the foregoing reasons and for the reasons set forth in the previously filed responses, applicant respectfully submits that the present claims are patently distinguishable from the teachings of Lawhon, Nakhmedov, and/or British '751 within the meaning of 35 U.S.C. §102 or 35 U.S.C. §103. Therefore, applicant respectfully requests that the examiner reconsider and withdraw the rejection of all the pending claims in this application over these teachings, and formally allow claims 27-30, 32-40, 44, and 49.

The present response is believed to be a complete and proper response to the Official action mailed on June 4, 2007. While it is believed that the present application is in condition for allowance, should the examiner have any comments or questions, it is respectfully requested that the undersigned be telephoned at the below listed number to resolve any outstanding issues.

In the event that this paper is not timely filed, applicant hereby petitions for an appropriate extension of time. The Commissioner is hereby authorized to charge the fee therefor, as well as any deficiency in the payment of the required fee(s) or credit any overpayment, to our deposit account No. 50-1147

Respectfully submitted,

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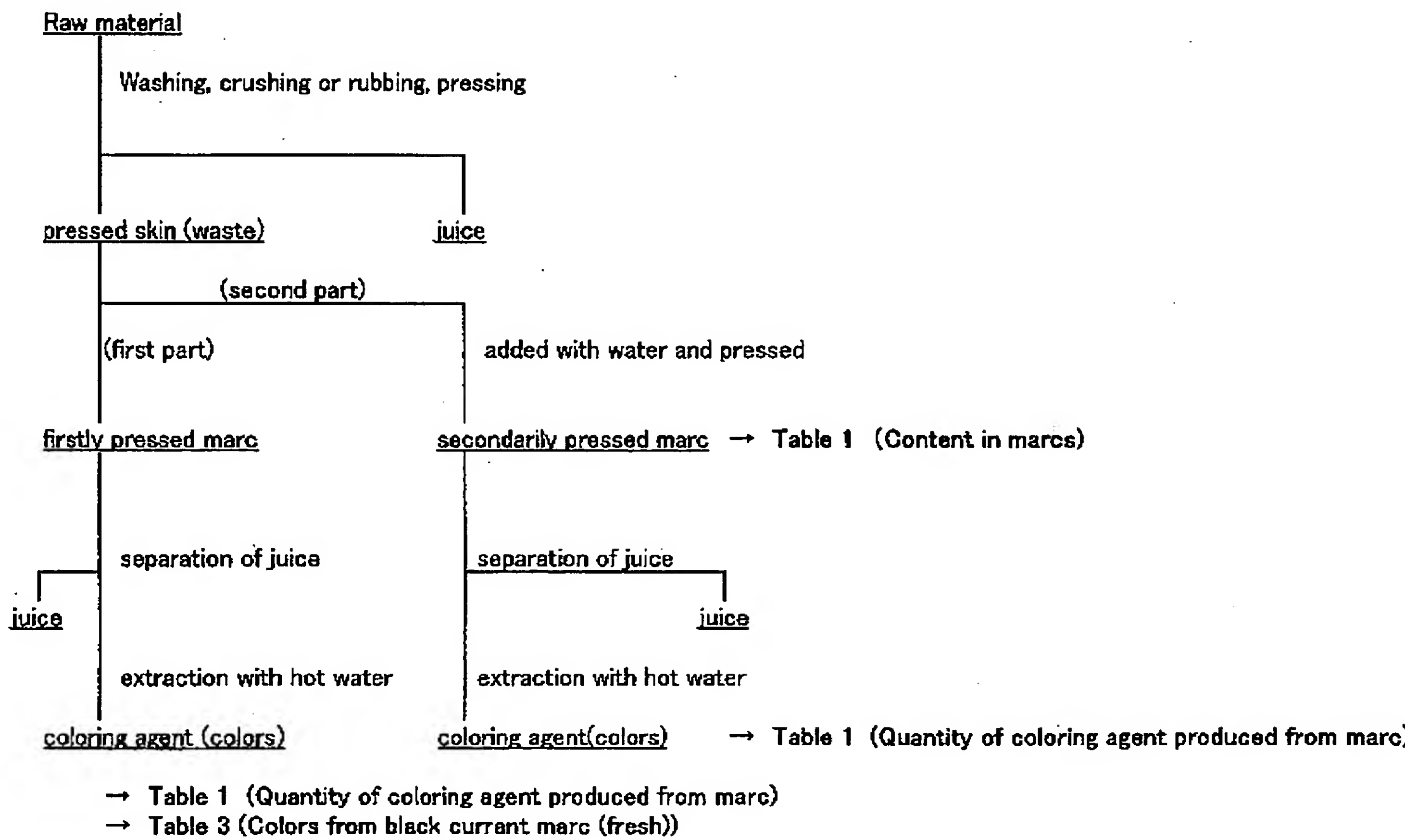
Attachments:

1. Scheme showing the process of purifying a coloring agent proposed in Nakhmedov (one page),
2. Boccorh *et al.*, entitled *Factors influencing quantities of sugars and organic acids and blackcurrant concentrates* in Z Lebensm Unters Forsch A (1998) 206; 273-278 (six pages) , and
3. Sanna Viljakainen, entitled *Reduction of Acidity in Northern Region Berry Juices* in ISBN 951-22-6435-8 (2003) (four pages).

Scheme which shows the production step of the coloring agent (colors) of Nakhmedov



Z Lebensm Unters Forsch A (1998) 206: 273–278

ORIGINAL PAPER

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Factors influencing quantities of sugars and organic acids in blackcurrant concentrates

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Abstract Use of endogenous non-volatile flavour components, i.e. sugars and organic acids, in fruit juice products is desirable. A study of 133 blackcurrant concentrates from three seasons examined variation in sugars and acids arising from storage of fruit at freezing or sub-ambient temperature, seasonal differences, geographical origin and choice of conventional thermal-evaporative or freeze concentration technology. Compared with freeze concentrates, conventional concentrates had significantly higher contents of total sugars and acids, notably malic acid, and higher fructose/glucose, lower malic/citric acid and similar sugar/acid ratios. Concentrates from frozen fruit generally had smaller amounts of fructose, total sugars and fructose/glucose ratios than those from fresh fruit, as well as less citric, ascorbic and total acids and lower sugar/acid ratios. Principal component analysis of 40 randomly chosen concentrates showed that variance is dominated by differences in fructose, total sugars and ascorbic acid contents and sugar/acid ratios. Geographical origin and concentration technology were major sources of variance but changes in post-production sub-ambient storage could not be excluded.

Key words Fruit · HPLC · Flavour quality · Sugar/acid ratios · Principal component analysis

Introduction

Sugars and organic acids, and their ratios, play important roles in the character and quality of the flavour of fruit juice products [1]. Commercially, endogenous sugars and acids have been supplemented with sugars and starch hydrolysates, as well as organic and mineral acids [1]. However, with changes in consumer demand and labelling it has become desirable to add only non-nutritive sweeteners to achieve acceptability.

Most blackcurrant drinks are produced from thermal-evaporative juice concentrates. During concentrate manufacture, major changes in fruit volatiles and Maillard browning reactions are favoured by increases in reactant concentrations and the elevated temperatures. Reactions between sugars and other components will continue in concentrates, albeit at reduced rates during subsequent storage at sub-ambient temperatures [2].

Alternative freeze concentration processes are now available, offering enhanced flavour quality [3]. Therefore, it is important to understand those factors that determine concentrate composition, particularly in terms of sugars and organic acids, in order to produce juice products that are perceived to have a natural character. Differences in post-harvest storage, the season of the fruit and processing technology all contribute to variation in juice products [4].

This work was aimed at establishing the significance of variation in the quantities of sugars and organic acids in thermal-evaporative blackcurrant concentrates from three seasons, prepared from either fresh or frozen fruit of various geographical origins, and comparing data with values for three freeze concentrates. This paper presents univariate and multivariate statistical analyses of data obtained by high-pressure liquid chromatography (HPLC) after sorbent extraction of the above-mentioned solutes from the concentrates.

Materials and methods

Samples. Blackcurrant concentrates ($n = 133$), processed from fruits of three seasons, were supplied by a UK manufacturer. A breakdown of samples from each season is provided in Table 1. All concentrates were stored at 4 °C in the dark.

Sorbent extraction. The method of van Horne [5] was adapted. Concentrate (1 ml) was diluted to 100 ml with deionized water, and acetic acid (AnalaR) and sucrose (AnalaR) were added, to 1 mg ml^{-1} final concentration, as internal standards. Solutions were neutralized with a few drops of 2 M NaOH (AnalaR). Cyclohexyl [CH; 1000 mg

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Table 1 Sugar contents of blackcurrant concentrates in 1989, 1990 and 1992 seasons. (*n* Total number of concentrates)

Season	Post-harvest storage/ Geographical origin (*n*)	Fructose (mg g⁻¹)	Glucose (mg g⁻¹)	Total sugars (mg g⁻¹)	Fructose/glucose ratio
1989	UK Fresh fruit (mean values) (13)	131.69	78.0	209.69	1.69
	SD	4.79	2.59	7.38	0.01
	SE	1.33	0.72	2.05	0.00
	UK Frozen fruit (32)	122.18	75.76	197.94	1.61
	SD	5.69	4.28	9.78	0.04
	SE	1.01	0.76	1.73	0.01
	New Zealand fruit (2)	169.86	112.48	282.34	1.51
	SD	1.17	0.77	1.94	0.00
	SE	0.83	0.55	1.37	0.00
1990	UK Fresh fruit (mean values) (34)	191.93	131.00	322.93	1.47
	SD	15.13	8.83	22.76	0.07
	SE	2.59	1.51	3.90	0.01
	UK Frozen fruit (10)	174.65	131.06	305.71	1.33
	SD	0.68	4.33	4.32	0.05
	SE	0.22	1.37	1.37	0.01
	Polish fruit (7)	163.20	122.40	285.60	1.33
	SD	6.76	4.34	11.10	0.01
	SE	2.55	1.64	4.19	0.00
	Imported fruit (7)	174.53	129.35	303.88	1.35
	SD	6.18	3.80	9.96	0.01
	SE	2.34	1.44	3.77	0.00
	New Zealand fruit (2)	151.11	108.43	259.54	1.39
	SD	1.57	0.45	2.01	0.01
	SE	1.11	0.32	1.42	0.01
	Freeze concentrates (3)	135.23	111.41	246.64	1.21
	SD	22.87	16.38	39.07	0.05
	SE	13.21	9.45	22.56	0.03
1992	UK Fresh fruit (mean values) (12)	169.88	137.53	307.41	1.24
	SD	25.17	13.53	29.31	0.20
	SE	7.27	3.91	8.46	0.58
	UK Frozen fruit (11)	153.56	135.54	289.10	1.13
	SD	13.35	11.43	20.40	0.10
	SE	4.03	3.45	6.15	0.03

(Varian)] and quaternary amine [SAX; 500 mg (Varian)] phase sorbent extraction columns were conditioned with column volumes of methanol and water and then mounted in tandem. Diluted concentrate (2 ml) was passed through at 2 ml min⁻¹ at 20 kPa vacuum. Filtrates were collected for sugar analysis. Top columns (CH) were then removed and SAX phases eluted with 2 ml of 0.2 M phosphoric acid (AnalaR) at 1 ml min⁻¹ at 20 kPa vacuum. Eluates were collected for analysis of organic acids.

For both groups of solutes identification was performed by preliminary HPLC analyses without internal standards, in which retention times of resolved peaks were matched with those of a number of standard pure solute solutions.

HPLC analysis. Sugars were quantified on Spherisorb NH_2 (5 × 240 mm) (Phase Separations, Clwyd, Wales) using acetonitrile/water (80/20) at ambient temperature and an ACS 740/14 evaporative mass detector (HPLC Technology, Macclesfield, England). Organic acids were determined on Spherisorb C18 (5 × 240 mm), eluting with 0.2 M phosphoric acid and monitoring effluent at 210 nm. Dry matter contents were calculated [6] and regression lines obtained using standard solutions of each identified component. Quantifications were performed in duplicate, calculating values using the internal standards.

Statistical analyses. Data were subjected to analysis of variance (ANOVA) using Minitab v8.2 and significance was inferred if $P < 0.05$. Principal component analyses were effected using Unscrambler II v3.1 (Camo A/S, Trondheim, Norway). This multivariate statistical procedure proceeds by extracting linear combinations of the original variables in data, to obtain a smaller number of variables (principal components, or factors). The first few factors explain the maximum of variance, whilst minimizing loss of information. Relationships between samples are described in two-dimensional spaces (Figs. 1–6), in which values (scores) on the first two principal components form the x and y coordinates (product spaces), respectively. The relationships between samples and the original variables are represented by plotting the distribution of variables (loadings) on the product spaces, to yield bi-plots.

Results

Tables 1 and 2 summarize available data on blackcurrant concentrates, including: season, geographical origin, post-harvest storage of fruit and concentration technology. Quantities of sugars and organic acids, ratios between individual sugars and between organic acids as well as total sugars/acid ratios were calculated. The significance of variation due to post-harvest storage and the geographical origin of the fruit was assessed. Differences were observed both between and within 1989 and 1990 concentrates for contents of fructose and total sugars and fructose/glucose ratios but not for glucose contents. Variation within 1989 and 1990 concentrates associated with the different geographical origins of the fruit were significant for all sugar

Table 2 Non-volatile acid contents of blackcurrent concentrates in 1989, 1990 and 1992 seasons

Season	Post harvest storage/ Geographical origin	Citric acid (mg g^{-1})	Ascorbic acid (mg g^{-1})	Malic acid (mg g^{-1})	Total acid (mg g^{-1})	Citric/malic acid ratio	Sugar/acid ratio
1989	UK Fresh fruit (mean values)	156.61	8.60	9.33	174.54	16.80	1.20
	SD	9.59	0.53	0.69	10.81	0.40	0.78
	SE	2.66	0.15	0.19	3.00	0.11	0.02
	UK Frozen fruit	148.58	6.14	8.89	163.61	16.71	1.21
	SD	11.66	0.70	0.86	13.22	0.41	0.11
	SE	2.06	0.12	0.15	2.34	0.07	0.02
	New Zealand fruit	171.81	7.50	10.66	189.97	16.12	1.49
	SD	0.44	0.04	0.06	0.54	0.05	0.01
	SE	0.31	0.02	0.05	0.38	0.04	0.01
1990	UK Fresh fruit (mean values)	142.37	12.44	12.96	167.77	10.98	1.92
	SD	12.50	2.34	3.77	17.64	1.57	0.21
	SE	2.14	0.40	0.65	3.03	0.27	0.04
	UK Frozen fruit	142.36	10.46	13.30	166.12	10.70	1.84
	SD	5.23	0.99	1.30	5.73	0.86	0.06
	SE	1.65	0.31	0.41	1.81	0.27	0.02
	Polish fruit	162.24	16.75	20.87	199.87	7.77	1.43
	SD	14.94	1.41	1.69	17.12	0.46	0.11
	SE	5.65	0.53	0.64	6.47	0.17	0.04
	Imported fruit	154.06	10.92	15.32	180.30	10.06	1.69
	SD	15.77	2.51	2.70	19.92	0.85	0.22
	SE	5.96	0.95	1.02	7.53	0.32	0.08
	New Zealand fruit	148.27	9.75	14.36	172.38	10.33	1.51
	SD	1.21	7.07 e^{-3}	0.22	1.42	0.07	0.00
	SE	0.85	5.00 e^{-3}	0.16	1.00	0.06	0.00
	Freeze concentrates	128.13	3.36	0.88	132.37	145.60	1.86
	SD	24.97	2.01	0.47	26.97	53.98	0.31
	SE	0.86	1.16	0.27	15.57	31.16	0.18
1992	UK Fresh fruit (mean values)	234.36	10.08	2.47	246.91	94.88	1.25
	SD	31.26	2.35	0.76	31.10	34.34	0.18
	SE	9.02	0.68	0.22	8.98	9.91	0.05
	UK Frozen fruit	163.97	6.40	1.81	172.18	90.59	1.68
	SD	18.36	2.06	0.95	19.68	68.69	0.28
	SE	5.54	0.62	0.29	5.93	20.71	0.08

parameters. In 1989 concentrates, variations in the contents of individual (citric, ascorbic and malic) and total acids in relation to post-harvest fruit storage were significant except for citric/malic acid and sugar/acid ratios. Differences due to geographical origin were significant for all parameters except ascorbic acid content. In 1990 concentrates, post-harvest fruit storage differences were significant in terms of all organic acid parameters whereas geographical origin was correlated with significant variation only for quantities of citric and total acids and not other acid parameters.

ANOVA was performed to study the effect of concentration technology. Significant differences in the amounts of fructose, glucose and total sugars as well as all acid contents and sugar/acid ratios but not the citric/malic acid ratio were observed. Two-way ANOVA indicated that the combination of post-harvest storage of fruit and concentration technology contributed significantly to the variation in the amounts of glucose and total sugars as well as the fructose/glucose ratio but not fructose contents and all acid values except malic acid content.

For 1992 concentrates, ANOVA indicated that the amounts of fructose and citric, ascorbic and total acids varied significantly in relation to post-harvest storage as did fructose/glucose and sugar/acid ratios.

Principal component analysis was also performed on the combined sugar and acid data sets from concentrates for each season. For the 1989 concentrates, samples scores on the first two components accounted for 97% variance but showed no clear differentiation within UK concentrates on the basis of post-harvest storage of fruit (Fig. 1). There was, however, clear separation between New Zealand and UK concentrates. Loadings indicated the former group of concentrates to have high levels of fructose, glucose and total sugars. Separation on the basis of fruit storage was apparent on the third component (Fig. 2). This factor accounted for only 3% of total variance, and was highly correlated with high fructose/glucose ratios in UK concentrates obtained from frozen fruit.

Figure 3 shows a principal component analysis bi-plot for 1990 concentrates: PC1 accounts for 66% of the total variance. Discrimination was based upon differences in the amounts of fructose and total sugars as well as the fructose/glucose ratios. Among UK concentrates, those made from frozen fruit had slightly lower (but significantly different) levels of these parameters. Factor 2, accounting for 18% of the variance, did not show clear separation of samples according to their known source of variation. Loadings, however, revealed that concentrates were separated on the basis of total acids, with citric and malic acids having large

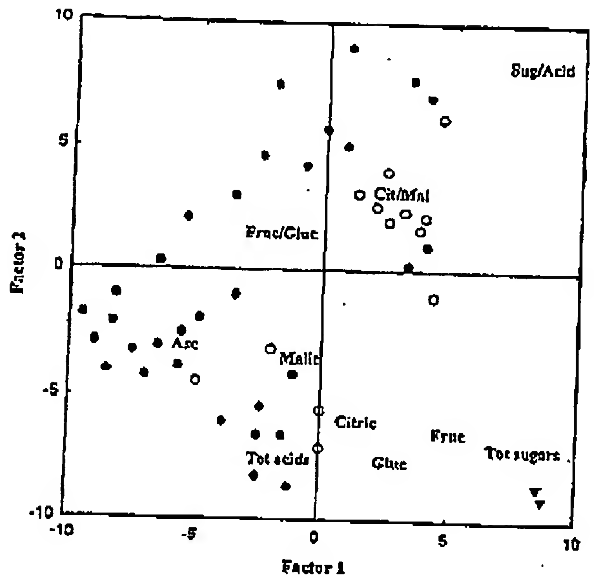

Fig. 1 Principal component analysis (*PCA*) bi-plot on the first and second factors, showing relationships between fresh (○), frozen (●) UK and New Zealand (▼) concentrates of the 1989 season, based on sugar and acid contents

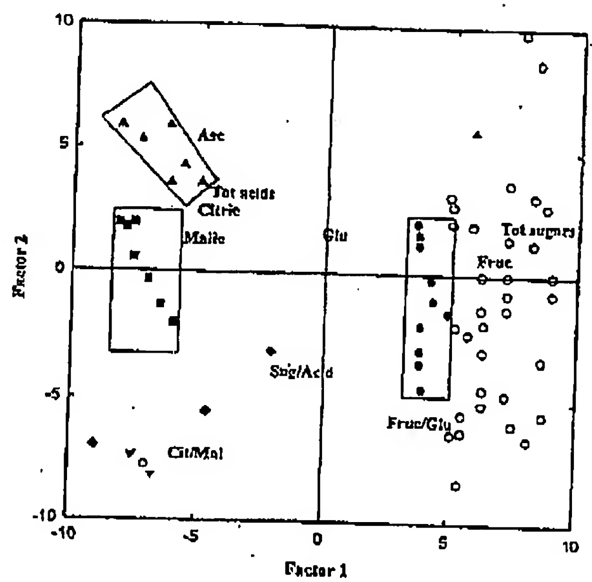

Fig. 3 PCA bi-plot on the first and second factors, showing relationships between fresh (○), frozen (●) fruit UK concentrates, imported (■), Polish (▲) and New Zealand (▼) and freeze (◆) concentrates of the 1990 season, based on sugar and acid contents

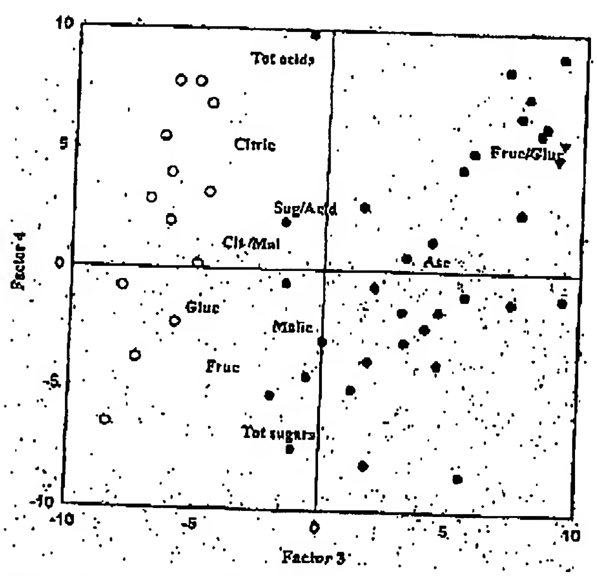

Fig. 2 PCA bi-plot on the third and fourth factors, showing relationships between fresh (○), frozen (●) UK and New Zealand (▼) concentrates of the 1989 season, based on sugar and acid contents

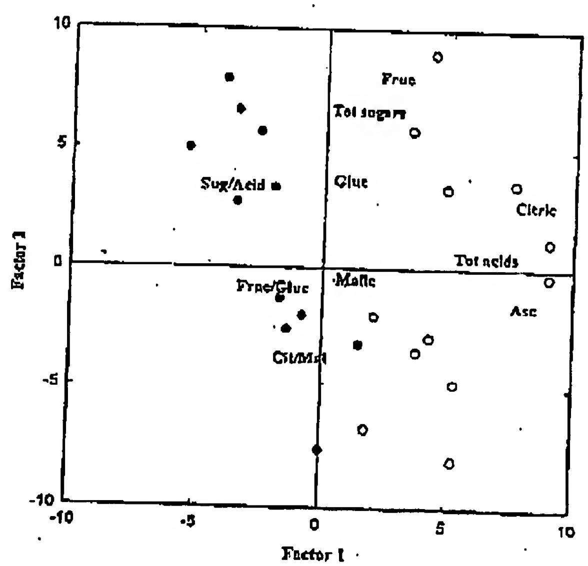

Fig. 4 PCA bi-plot on the first and second factors, showing relationships between fresh (○) and frozen (●) concentrates of the 1992 season, based on sugar and acid contents

influences. Polish concentrates were discriminated on the basis of containing relatively large amounts of total, citric and malic acids, whereas those from New Zealand contained relatively little. UK fresh-fruit concentrates were widely distributed on the basis of this component.

For 1992 concentrates (Fig. 4) PC1 explained 77% of the variance, clearly separating concentrates manufactured from fresh and frozen fruit on the basis of amount of total acids, mainly citric and ascorbic acids. In contrast, PC2 (14% of the variance), separated concentrates on the basis

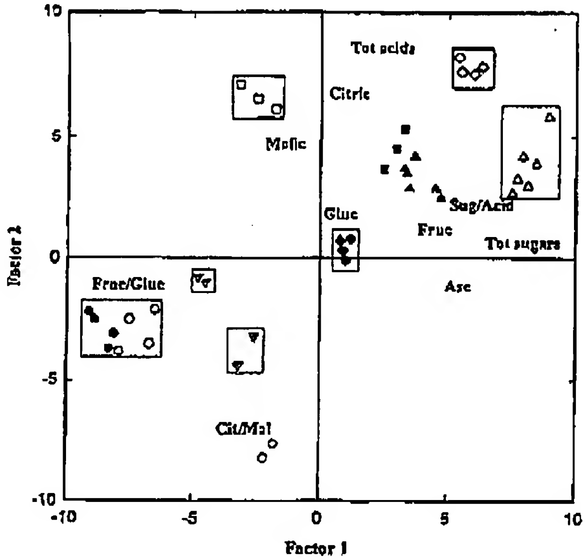

Fig. 5 PCA bi-plot on the first and second factors, showing relationships between season, post-harvest storage, geographical origin and concentration technology on sugar and acids contents of blackcurrant concentrates. (○ 1989 UK fresh fruit, ● 1989 UK frozen fruit; ▽ 1989 New Zealand, △ 1990 UK fresh fruit, ▲ 1990 UK frozen fruit, ■ 1990 imported fruit, □ 1990 Polish, ▼ 1990 New Zealand, ○ 1990 freeze samples, ◇ 1992 UK fresh fruit, ◆ UK frozen fruit)

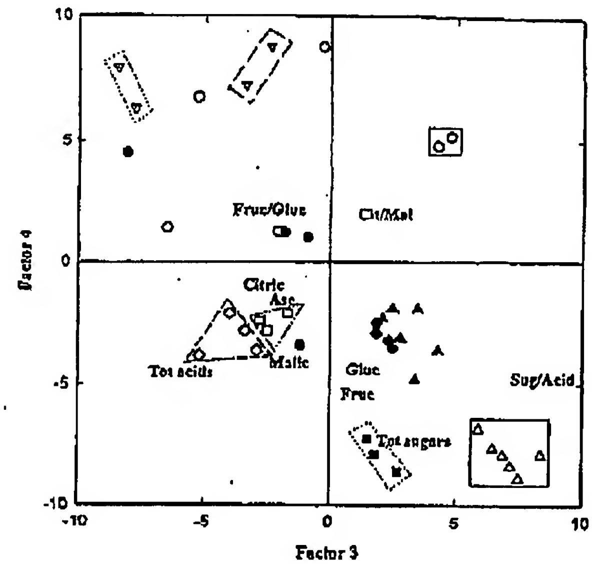

Fig. 6 PCA bi-plot on the third and fourth factors, showing relationships between season, post-harvest storage, geographical origin and concentration technology on sugar and acids contents of blackcurrant concentrates. See legend to Fig. 5 for explanation of symbols

of their fructose and total sugar contents. This was not correlated with any known source of difference.

The results of principal component analysis on the data of 40 randomly selected concentrates from all three seasons, varying in post-harvest storage, geographical origin and concentration method, are indicated in Fig. 5. Factor 1, 37% variance, grouped UK 1990 and 1992 concentrates as well as imported concentrates and could be related to differences in fructose, total sugars and ascorbic acid contents and sugar/acid ratios. Factor 2 (19% variance) discriminated UK 1990 and 1992, Polish and imported concentrates on the basis of citric and total acid contents. Inspection of scores on PC3, 8% variance (Fig. 6), separated concentrates primarily on the basis of differences in sugar/acid ratios.

Discussion

From this study it is apparent that freeze concentrates have significantly lower contents of individual and total acids compared with conventional thermal-evaporative concentrates made from fresh berries, but similar values for citric/malic acid ratios. The effect of post-harvest storage of berries is also clear: thermal concentrates from fresh berries have significantly higher ($P < 0.050$) ascorbic acid contents (11.1–12.4 mg g^{-1}) than those from frozen fruit (9.8–10.0 mg g^{-1}). Fresh-fruit thermal concentrates also have significantly higher ($P < 0.050$) citric/malic acid ratios, at 11.6–12.5, than those from frozen fruit, at 10.0–10.5, which is a reflection of increases in malic acid content from 10.4–12.7 to 13.5–14.5 mg g^{-1}. Such differences associated with post-harvest fruit storage were significant for total acids and sugar/acid ratios.

Although there were significant variations in fructose and total sugars contents associated with use of frozen fruit, glucose contents were similar. When the combination of season and post-harvest storage was considered, neither variation in fructose nor total sugars content was significant. In all three seasons the fructose/glucose ratio was significantly related with post-harvest fruit storage. When the geographical origin of the fruit was considered, only variations in the quantities of citric and total acids were significant.

Of the individual seasons, it was apparent that 1989 thermal concentrates could be discriminated into two clusters on the basis of post-harvest fruit storage, with those from fresh berries containing more fructose and, consequently, having higher fructose/glucose ratios. Concentrates from New Zealand frozen fruit contained more of all sugars, but had lower fructose/glucose ratios compared with those made from UK berries. Results for 1990 concentrates were similar; however, 1992 concentrates had lower fructose/glucose ratios compared with those from preceding years.

Variation in the sugar contents of thermal concentrates can have two origins: differences in fruit and its post-harvest storage prior to processing; and variation in the rates or extent of post-processing reactions during storage at sub-ambient temperatures. Differences in blackcurrant composition can arise from factors related to genotype,

soils and agronomic practice. During post-harvest storage of fruit, concentrate manufacture and the subsequent storage at sub-ambient temperatures, glucose and fructose will participate in the initial stages of Maillard reactions, forming Amadori and Heyns intermediates. In such reactions, glucose, the preferred substrate [7], is slowly converted to 3-substituted furans and furaneols [8–10]. Although 3-(2*H*)-furanones may theoretically be formed, the acidity of the concentrates does not favour such reactions [11].

Prolonged storage of concentrates, such as those of the 1989 season, was predicted to reduce ascorbic acid contents. Thomson and Fennema [12] suggested losses of up to 30% in ascorbic acid content during freezing, storage and subsequent thawing with many fruits, a view not shared by Loeffler [13] or Sulc [14]. Thomson and Fennema [12] did, however, concede that a low pH and low oxygen content may restrict losses to $<5\%$. In the present study, 1992 concentrates had particularly high contents of all acids and relatively little variation ($<3\%$), which could be ascribed to the use of fresh or frozen fruit. Differences between fruits are important in determining ascorbic acid losses [15], but cultivar differences could not be concluded from this study since single-variety blackcurrant concentrates were not available.

The reduced contents both of sugars and acids in freeze concentrates compared with those conventionally prepared can be ascribed to losses in ice formed during the freezing process.

Although the composition of fruit juice products may originate in differences in the original fruit, blending both of fruit, prior to processing, and concentrates is commonly done by manufacturers. The processing plant itself is a major factor that contributes to differences in fruit concentrate composition [16], but differences in post-harvest fruit storage influence respiratory activity in berries [17] and nutrient loss [18]. The absence of sucrose in concentrates may be the result of inversion, recorded to occur in blackberry [2] and apple [19, 20] concentrates. In this study glucose contents may have decreased during post-processing storage at sub-ambient temperatures, whereas the major effect in blackberry concentrates was on acid content [2]. The highest sugar/acid ratios in concentrates was observed in those made from fresh and frozen fruit in 1989; the lowest were observed to occur in 1990 concentrates, most likely a reflection of the prevailing weather at the time of harvest.

In summary, any conclusion that fresh fruit yields the best quality juice cannot be extrapolated to considerations of acids and sugars in blackcurrant concentrates [21]. Differences in the amounts of individual and total acids will influence perceptions of the flavour, character and quality of juice products. Differences in sugar contents also influence product mouthfeel, as exogenous sucrose contributes more compared with endogenous glucose or fructose [1]. The natural non-volatiles in juice products have not been extensively studied. They may, however, play important roles in determining sensory factors such as mouthfeel and temporal characteristics of flavour [22], suggesting that more studies of the influences of differences is warranted in the search for juice products perceived to be of premium quality by consumers.

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Reduction of Acidity in Northern Region Berry Juices

Sanna Viljakainen

Dissertation for the degree of Doctor of Science in Technology to be presented with due permission of the Department of Chemical Technology for public examination and debate in Auditorium KE 2 (Komppa Auditorium) at Helsinki University of Technology (Espoo, Finland) on the 15th of May, 2003, at 12 noon.

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Abstract

Northern region berries may be used for wide variety of product alternatives as well as serving as a supply of many nutritionally valuable components. However, there are only rare, fragmentary and inconsistent scientific data available on the chemical composition of northern region berries. Therefore, comprehensive information was gathered on organic acid and soluble sugar concentrations of juices of six wild berries (bilberry, lingonberry, cranberry, cloudberry, red raspberry, black crowberry) and five cultivated berries

(black currant, white currant, red currant, gooseberry (red), strawberry) all grown in Finland. The main acids of the berry juices were invariably citric and malic acid even though their concentrations varied widely from one berry variety to another (2.9 - 16.2 g/l and 3.3 - 24.7 g/l, respectively). In addition, juices of lingonberry, cranberry, cloudberry and black crowberry contained benzoic acid (0.1 - 0.7 g/l). The main sugars of the investigated berry juices were fructose (18.0 - 57.2 g/l) and glucose (22.2 - 50.0 g/l). Most of the berries contained also sucrose (0.2 - 5.1 g/l). The data enable direct comparison of northern region berries and underline the wide variation in their organic acid and soluble sugar content, which offers possibilities for the production of numerous sensory profiles. Accordingly, the selection of the right berry for individual purposes is enhanced.

Due to their acid and sugar composition, fermentation of northern region berry juices into wines faces challenges that are not normally met when using grape juices. In berry juices the fermentations should maintain or alleviate the often rich berry aroma under conditions where the content of organic acids is high and that of fermentable sugars low. Prior to fermentation the juices have to be diluted and sugar has to be added. This causes significant weakening of the aroma and body of the wine. To reduce the acidic mouthfeel of grape wines malolactic fermentation (MLF) is widely used. However, it is not known whether MLF is applicable to modifying the acid composition of berry juices. Therefore, acid conversion by MLF by *Oenococcus oeni* was studied to improve the usability of northern region berry juices. During MLF at low pH values (pH < 4.5), malic acid was always degraded first to completion without consumption of sugars or citric acid. After the exhaustion of malic acid the degradation of both citric acid and glucose were initiated simultaneously. Thus, it is concluded that by MLF, selective conversion of malic acid to lactic acid can be achieved without loss of sugar, also in berry juices. Sequential utilization of

substrates by MLF thus enables a multitude of compositional changes in acidic juices. Control of duration of the fermentation is essential when acid reduction without loss of sugar should occur.

The most problematic compound with reference to winemaking from lingonberry is benzoic acid, which contributes to the acidity of the berry. As a microbicidal compound, benzoic acid also prevents fermentation of lingonberry juice. Thus, the known pH-dependent ability of *Saccharomyces cerevisiae* yeast to uptake benzoic acid from solutions was applied. By suspending 15 - 20 % (w/w) of the yeast for 10 min in undiluted lingonberry juice, the benzoic acid concentration was reduced by 75 - 91 %, titratable acids by about 14 % and pH increased by 0.1 units. The resulting undiluted juice was successfully fermented with a new yeast inoculum. Thus, yeast may be used as a selective absorbent to remove a certain fermentation-hindering component from the juice. These results offer new insights into berry juice fermentation.

Accordingly, MLF represents a new, promising means for acidity reduction of northern region berry juices and berry wines without a significant loss of their natural sugar content. Also, the benzoic acid uptake by the yeast was proven to be effective. By these new methods, the critical inhibitors of the further processing of the juices can be eliminated and thus it is possible to facilitate the development of various berry products of northern regions.

This thesis consists of an overview and of the following 5 publications:

1. Viljakainen, S., Visti, A. and Laakso, S., 2002. Concentrations of organic acids and soluble sugars in juices from Nordic berries. Acta Agriculturæ Scandinavica B 52, pages 101-109. © 2002 Taylor & Francis. By permission.
2. Viljakainen, S. and Laakso, S., 2000. The use of malolactic *Oenococcus oeni* (ATCC 39401) for

deacidification of media containing glucose, malic acid and citric acid. European Food Research and Technology 211, pages 438-442. © 2000 Springer-Verlag. By permission.

3. Viljakainen, S. and Laakso, S., 2002. Acidity reduction in northern region berry juices by the malolactic bacterium *Oenococcus oeni*. European Food Research and Technology 214, pages 412-417. © 2002 Springer-Verlag. By permission.
4. Viljakainen, S., Visti, A. and Laakso, S., 2003. Malolactic and alcoholic fermentations in black currant juice. Die Wein-Wissenschaft, in press. © 2003 by authors and © 2003 Fachverlag. By permission.
5. Visti, A., Viljakainen, S. and Laakso, S., 2003. Preparation of fermentable lingonberry juice through removal of benzoic acid by *Saccharomyces cerevisiae* yeast. Food Research International 36, in press. © 2003 Elsevier Science. By permission.

Keywords: northern region berries, organic acids, soluble sugars, acid reduction, malolactic fermentation, *Oenococcus oeni*, yeast uptake, alcoholic fermentation, berry wine, sensory quality